## Wahlsten, Jennifer L.

From: Maciej Czerwinski [mczerwinski@xenotechllc.com]

Sent: Thursday, September 18, 2008 10:51 AM

To: Wahlsten, Jennifer L. Subject: XenoTechniques

## Dear Jennifer,

Information contained in the XenoTechniques Vol. 1, No. 1 was disseminated to the public during 12 - 16 October 2003 ISSX Meeting in Providence RI. The Figures 3, 4, 6, 7, 8, 9 and 10 published in XenoTechniques were originally presented in our poster at that meeting. Please see the attached pdf. At the same meeting additional information regarding properties of Fa2N-4 cells was presented by scientists from Roche in the scientific poster number 249.

A pdf version of a final version of XenoTechniques Vol 1., No. 1 was created on July 19, 2004 and has subsequently been used to promote the cell line.

Please feel free to contac us again if we could be of further assitance.

With regards,

Maciej Czerwinski, Ph.D. Principal Scientist - Cell and Molecular Biology 913 227 7113



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thate at primary extracts of Junion hopstory, for. The magnitude of staction of CYP3A4 in Fa2N-4 cells colleged in 12-, 24- and 96-ell places was comparable to that in 6-teell places, which bodgs well r the development of uninitarized, higher throughput assays. In manny, PAIN-4 cells demonstrated hoputors tellak responsiveness CYP magnin indusest and present a promising alternative to many enturing of human hepatocytes for embasting the erromeinduction of CYP2B6, established in three experiments, dementationed the consistency of the system's performance (Figure 2). The CYP2C19 incinity of Sampleservoin hydroxyleso was not induced by treatment of the cells with riferentia.

get generic facesis. Recently, behinder Tennedages (Worwick, 81) intenditional branch associate by remindenting gramas, waiting with DNA of the Tanadeges of sistems (NASO view. In termining self-ural, DNA of the Tanadeges of sistems (NASO view.) and the self-sional process of the SNA of the S The ability of drug candidates to induce CVP earymet, paracularly CVP IA2 and CVP IA4, it exemunely evaluated in wire with primary subtrees of human beyones (as. The rapph) of burnes livers a rabbit or abu propose to increasingly limited and their response to naw for abuly propose to increasingly limited and their response to naw themself entitles (DCCE) in viriable due to primartness can incremental themself entitles (DCCE) in viriable due to primartness can incremental themself entitles (DCCE).

alls could be assessed based on enginentic activity. In addition, we harecorrized the industribley of targer CVPs in Fa2N-4 spilling inhiple-well formass and a nationarized, higher stroughput archeolon.

The FAIN-4 cells were placed using propriouse plaining and maintenance rodio operation of authoritie beforeware of the offi-tenance of the control of the cells of the cells of the Wingoo. (Colorine Technologies) on Justicevier, control with Wingoo. (Colorine Technologies) blook does, CAL at JTC. 29. CO2, 1954; lamonies, "Typianis, calcular of FAIN-4 cells were givens to condition; in swell places doesd with (IO) and comprobel or 20 plus from the Tabust and metabook with (Typicolife technical for fullament for Tabust and metabook with (Typicolife technical for Summary of the LC/MS/MS analytical methods

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(CYPIA2). The magnitude of industron in Fa2N-4 cells was comparable to that of primary cultures of benum hepstocytes in 60-mm debes (Table 1). The dozed colluses (Figure 1) In multiple experiments, reterrent of Fa2N-1 cells with 20 jubl relations CYP2Bs, CYP2C9, and CYP2C19 for up to 6 hours cells with 100 µM emephasole caused, on average, a 20-fold induction of phonaectin O-doally/lase nereaso in diciofonac 4'-hydroxylaza (CYP2C9) softway and a 51-fold increase is midazolam 1'end up to 2 hours for CYP3A4 in vehicle and inducer FallN-I bepatocytes was inve Activity, and inducibility of CYP1A2, CYP2B6 CYP2C9, CYP2C19 and CYP3A4 in immortalized dross late (CYPSA4) activity. Treatment of Pa2Nabolitos was linear over time for CYPIAZ reclium and followed by measurement and followed by measurement and CYP-special and CYP-specia on average, in a 25-fold increase roxylate (CYP2B6) activity, a 2.6-fole

prinary onliners of hepatocytes (LeCture et al., 2000). Time dopendence tank of inducion of CYPIA2 and CYP3A4 indicated maximal response at 72 hours, in which the Fa234d cells narrowed responsiveness of hepatocytes in prenary cellures. The emptranele concentration response of CYPIA2 in Fa2N-4 cells reached a peak response at 50 µM. In order to assess whether the FaZN-4 cells can be had mountal response at 20 µM riftenpin (Figure 3).
For both CYP cato, nets, immertalized hepatoes as while the concentration response care of CYP3A4 hibited peak response at the same concentration as

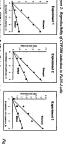
the rano across different plate formats, while induction of CYPIA2 in %-nell plate format (expanded in a single experiment at hour incohairen with phonocoun) was lower in comparison to 6-well used in anniaturaced screening sate, we compared industrially of CYPIA3, CYP286, CYP2C9, and CYPIA4 in 6, 12, 24 and 95-well plates. The industriality of CYP286, CYP2C9, and CYP3A4 was Figure 5: CFP induction in Fa2N-4: multiple plate formats

compounds in 24-well place ontoures. The system corrective differentiated known pregnance. recopied agrees from induces soring through different nuclear receptor years were such as and hydrocarbon receptor (em. phrasole, 3-suchlydelolandurene) (Figure 6).

ed with CYP Figure 2: Reproducibility of CYP2B6 induction in Fa2N-4 cells Figure 1: CYP induction in Pn2N-4 cells CYP 142 - Phon -CYPICS - Debe

edls in primary cultures, such un extra more more and offer significant advantages over more awards, scarce bepaneyes: The factor developed to daying understand bepaneyes:

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Rebust growth, ease of handling and good responsiveness of Fa2N-4 cells in 12, 24 and 95-sell plates bodes well for a rapid development of a higher throughput industrien content development of a higher throughput industrien content pendetable manner that minist bepasteyres in magnitude of response, consentation response to prototypent inducers, and the time course of induction. Since the insuscritized legislacy, see on the exceptions of and not readily, available, they constitute a reproducible, well-abaroneerized system for response to take.





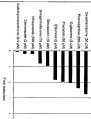
Figure 4: Time course of CYP Induction in Fu2N-4 orth

Indicated by Proceedings of The Internal Interna Politico II., Notan A. Haratiso O., Candi K. Daliban R. and Patitison A. (2001). Digression and regulation of a studiented PMD anguses in princip exhibits of

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Figure 6: Ernination of effect of known engine inducers on CYP3A4 agricle; in Fa2N-4 colle

Dexamethasone (50 µM) Phynobarbital (500 µM) Simvactate (10 µM) Phenytoin (50 µM) Cigitazone (2 µM) Etavirenz (2 µM) Rifampin (20 µM)



able 1: CYP induction in vitro in Fa2N-4 cells versus bumon hapotocytes

CASTO

2.5 fold (2.0-3.9 fold) 5.1 614 (4.0-6.9 fold) 2 % feed (1 6-2 8 fold) 20 fold (9.3-29 fold) Fa2N-4 cells

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